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Effects of nutrient supplies on the growth rates of planktonic  
bacteria in a bay of the Uwa Sea, Japan

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Running head: Bacterial growth rate in a bay

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## ABSTRACT

In the oligo- to meso-trophic waters of Uchiumi Bay, Japan, there is an intermittent physical event which supplies nutrients to the sea: bottom intrusion of deep, cool, nutrient-rich water that slides through just above the continental shelf. To clarify effects of nutrient supply by this bottom intrusion on bacterial growth, we followed seasonal changes in bacterial growth rates and conducted nutrient amendment experiments with single or mixed-nutrient additions of nitrogen and phosphorus. In addition, we examined the importance of organic matter supply for bacterial growth rates using glucose. Bacterioplankton bulk growth rates ranged between 0.37 and 1.86 d<sup>-1</sup>, with relatively high growth rates during the period of strong thermal stratification. Neither positive nor negative effects on bacterial growth rates were detected by amendments of nutrients or glucose. A significant positive relationship ( $p < 0.001$ ) between cell density and production of bacteria was found, though the regression slope of bacterial cell density on bacterial production was relatively low (0.34). Our results suggested that bacterial growth rates in Uchiumi Bay are nearly saturated at ambient levels of nutrients and/or organic matter, and that bacterial abundance is subject to a relatively weak bottom-up control.

Key words: bacterial growth rate, nutrient limitation, bottom-up control

## INTRODUCTION

Planktonic heterotrophic bacteria constitute a large portion of the planktonic biomass in both marine and freshwater ecosystems (Cho & Azam 1990, Geller et al. 1991, Kawabata & Nakanishi 1996, Simon et al. 1992). Thus, bacterial utilization of inorganic and organic matter and, resultant production of bacterial biomass may account for a substantial part of material cycling in an aquatic system. Bacterial production depends on bacterial abundance, cell size and growth rate. It has been considered that abundance and growth rates of bacteria in natural aquatic environments are susceptible to subtle changes in environmental conditions. Studies on the seasonal dynamics of bacterial abundance frequently show a relative constancy of cell density over time (Azam et al. 1983, Pace 1988). In contrast, bacterial growth rates vary depending on environmental variables: temperature, nutrient and energy supplies as bottom up control, and grazing and viral lysis as top down control (Church et al. 2000, Sala et al. 2002). Bacterial growth rates are therefore a useful measure in elucidating environmental factor(s) which may cause changes in bacterial production. There is a significant



1 correlation between bacterial growth rates and phytoplankton biomass (Cole et al. 1988,  
2 White et al. 1991). Thus, it is well accepted that bacterial growth rates are closely  
3 related to the productivity of an aquatic ecosystem.

4 Owing to their bulk in nucleic acids and proteins, the nitrogen and phosphorus  
5 requirements of bacteria are large. This necessitates bacterial cells to maintain low  
6 intracellular C:N:P ratios (Goldman et al. 1991, Tezuka 1990). The amount of available  
7 nutrients can therefore constrain bacterial growth rates (Caron et al. 2000, Hoch &  
8 Bronk 2007, Rivkin & Anderson 1997). Evidences have been accumulated that bacteria  
9 in the Atlantic Ocean, the Mediterranean Sea, Baltic Sea are limited by inorganic  
10 nutrients, mainly phosphorus (Caron et al. 2000, Pinhassi et al. 2006, Rivkin and  
11 Anderson 1997). Also, nutrient limitation of bacterial growth in lakes has been  
12 intensively studied (Elser et al. 1995, Morris and Lewis 1992, Jansson et al. 1996).  
13 However, such information is relatively limited for marine coastal waters especially in  
14 Western Pacific Ocean.

15 The Uwa Sea is located along southwestern coastal area of Shikoku Island, Japan.  
16 The trophic state of the sea has been considered meso- to oligo-trophic (Tomaru et al.  
17 2002). Within this system, there is an intermittent physical event which supplies  
18 nutrients to the sea: the bottom intrusion of deep, cool, nutrient-rich Pacific water which

slides just above the continental shelf (Kaneda et al. 2002 a & b). Large amounts of nitrate and phosphate supplied by bottom intrusion are the major nutrient source for phytoplankton primary production in the sea (Koizumi 1991, Koizumi & Kohno 1994, Koizumi et al. 1997, Takeoka et al. 2000). Since inorganic nitrogen (Rivkin and Anderson 1997) and/or phosphorus (Caron et al. 2000, Pinhassi et al. 2006, Rivkin and Anderson 1997) supplies stimulate bacterial growth in marine environments, bottom intrusion may also serve as an important supply of nutrients for bacteria in the Uwa Sea. The importance of nutrients supplied through bottom intrusion on bacterial growth rates remains to be resolved.

In the present study, to elucidate the effects of nutrient supply by the bottom intrusion on bacterial growth rates, we followed seasonal changes in bacterial growth rates and conducted nutrient amendment experiments in a bay of the Uwa Sea. To identify which nutrient is important for accelerating bacterial growth rates, we made single or mixed-nutrient addition experiments. We also examined the importance of organic matter supply for bacterial growth rates.

## MATERIALS AND METHODS

# 1 Monthly monitoring on physicochemical and biological parameters

2           There are two major physical events in the Uwa Sea: *kyucho* and bottom  
3 intrusion both of which occur mainly in summer. The former is an intrusion of surface,  
4 warm, oligotrophic water from the south of the Bungo Channel to the west coast of  
5 Shikoku Island (Takeoka et al. 1993, Takeoka & Yoshimura 1988). Bottom intrusion  
6 consists of deep, cold, nutrient-rich water that flows just over the continental shelf  
7 (Kaneda et al. 2002 a & b, Takeoka et al. 2000), serving as the major nutrient input to  
8 the coastal areas (Koizumi 1991, Koizumi & Kohno 1994, Koizumi et al. 1997, Takeoka  
9 et al. 2000). While the effect of *kyucho* on phytoplankton growth is negligible, diatom  
10 blooms are observed to be promoted by nutrient inputs by bottom intrusion (Koizumi &  
11 Kohno 1994, Koizumi et al. 1997). Thus, the trophic status of the Uwa Sea is  
12 temporarily changed when a bottom intrusion happens, and the dominant biological  
13 matter cycling may be changed by a shift of the dominant food linkages from a  
14 microbial food web to a herbivorous food web due to the occurrence of bottom intrusion  
15 (Nakano et al. 2004).

16           The present study was conducted at the station Ub (Fig. 1, 33° 2' N, 132° 28' E;  
17 water depth, ca 53 m) in Uchiumi Bay located in Iegushi, Uchiumi Village, Ehime  
18 Prefecture, Japan (Nakano et al. 2004), which opens to the Bungo Channel within the

Uwa Sea (Fig. 1). The Channel is strongly affected by an oligotrophic Kuroshio front (Takeoka et al. 2000). There are no rivers or streams flowing into the Bay so there is negligible effect on water quality by nutrient loading of inflows. The sampling station, Ub was located in the center of a pearl oyster culture farm. Water samples were collected monthly using a 6 L Van-Dorn water sampler from 0, 2, 5, 10, 15, 20, 30, 40 and 50 m depths from June 2002 to June 2003. Vertical distribution of water temperature was determined with a Chlorotech profiler (Arec Electronics Co., ACL-208-DK). To monitor the occurrence of bottom intrusion, the water temperature was measured every 30 min with a thermistor chain at 2 and 60 m depths at the station Ut (Fig. 1).

A 100 ml portion of the water sample was filtered through a 0.2  $\mu$ m Nuclepore filter, and the filtrate was used for measuring the concentration of dissolved inorganic nitrogen ( $\text{DIN} = \text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$ ) and soluble reactive phosphorus (SRP) using an AutoAnalyzer 3 (BRAN-LUEBBE co. Ltd.).

Another 100 ml portion of the water sample was filtered through a GF/F filter (Whatman) precombusted at 450  $^{\circ}\text{C}$  for 2 hours, and the filtrate was used to measure the concentration of dissolved organic carbon (DOC) using a total carbon analyzer (TOC-V, Shimadzu Co. Ltd.). DOC measurements were conducted between February 2003 and

1 August 2003.

2 To determine chlorophyll *a* concentration, a 100 ml water sample was filtered  
3 through a 0.2  $\mu$ m Nuclepore filter to retain seston. Each filter retaining seston was taken  
4 into a glass test tube, and 6.5 ml of N, N-dimethylformamide (DMF) was added to  
5 extract chlorophyll *a*. The amount of chlorophyll *a* thus extracted was determined using  
6 the fluorometric method (Moran & Porath 1980).

7 For enumeration of heterotrophic bacteria, a water sample (100 ml) was fixed  
8 with glutaraldehyde at a final concentration of 1%. Bacteria were stained with 4'6 -  
9 diamidino - 2 - phenylindole (DAPI) and filtered on black polycarbonate filters (0.2  $\mu$ m  
10 pore size, Porter & Feig 1980). At least 300 bacterial cells, in most cases > 400 cells on  
11 the filter were counted using an epifluorescence microscope under UV excitation.

12

### 13 **Nutrient amendment experiment**

14 We used the dilution experiment for determining nutrient limitation on bacterial growth  
15 rates. The method proposed by Kirchman et al. (1982) and used for studies on nutrient  
16 limitation on bacterial growth in various environments (Felip et al. 1996, Middelboe et  
17 al. 2002, Morris & Lewis Jr 1992, Rivkin & Anderson 1997) was followed. Water  
18 sample collected from 10 m depth, where high abundances of phytoplankton and

1 bacteria have been previously observed (Ichinotsuka et al. 2006, Katano et al. 2004,  
2 Katano et al. 2005) was used. The sample was subjected to size-fractionations as  
3 follows: 0.8  $\mu\text{m}$  Nuclepore filter for removing consumers of bacteria such as protists  
4 under negative pressure at 0.05 MPa, and 0.2  $\mu\text{m}$  Gelman culture capsule filter for  
5 removing all planktonic organisms using gravity. We discarded the first one liter of the  
6 filtrate of Gelman culture capsule filter to minimize the contamination of dissolved  
7 organic matter released from the filter. We diluted the  $< 0.8\mu\text{m}$  filtrate into the  $< 0.2 \mu\text{m}$   
8 filtrate at a dilution level of 0.1, and 200 ml portions of the diluted water sample were  
9 poured into twenty 250 - ml polycarbonate bottles. In our microscopic observation, cell  
10 size of heterotrophic nanoflagellates was mostly 4 - 5  $\mu\text{m}$ . Thus, our manipulation  
11 removed most protist cells from the seawater. Moreover, the removal of grazers and the  
12 dilution of the sample resulted in a reduction in the amount of grazing on bacteria.  
13  $\text{NH}_4\text{Cl}$  ( $\text{NH}_4$ ) as inorganic nitrogen,  $\text{NaH}_2\text{PO}_4$  ( $\text{PO}_4$ ) as inorganic phosphorus and  
14 glucose as organic matter were added to each of four bottles to attain final  
15 concentrations of 15  $\mu\text{mol N l}^{-1}$ , 1  $\mu\text{mol P l}^{-1}$  and 30  $\mu\text{mol C l}^{-1}$ , respectively. Mixed  
16 supplements were also made. Thus, our experiment was carried out in four replications.  
17 In that  $\text{NH}_4$  and  $\text{PO}_4$  were added to four bottles. The remaining four bottles served as the  
18 control. The 20 bottles thus prepared were incubated at 10 m depth in the near shore

waters of Uchiumi Bay for 24 hours. A recent study on bacterial production in a marine environment has shown that, although the incubation duration of one day was enough to detect changes in bacterial production by the leucine uptake method, changes in bacterial abundance could not be discerned during the period (Pinhassi et al. 2006). However, we also conducted the experiments using 48 hours incubation to determine bacterial growth rate between June 2002 and February 2003, and found that the bacterial growth rates derived from 24 hours incubation were not significantly different from those of 48 hours incubation (data not shown). So, we decided to use bacterial growth rates determined in experiments of 24 hours incubation. At time zero and at the end of the incubation, subsamples for the enumeration of bacteria were taken and fixed as mentioned above. The growth rate,  $\mu$ , was calculated from the equation,  $\mu = \ln(N_f / N_0) / t$ , where  $N_0$  and  $N_f$  are cell densities at time zero and after 1 day of incubation, respectively, and  $t$  is the incubation period. One-way ANOVA was used to test for difference among treatments: subsequent multiple comparisons were performed using Tukey's test with a statistical significance level of  $p < 0.05$ .

## **Estimation of bacterial production**

Bacterial production, BP ( $\mu\text{g C l}^{-1} \text{ d}^{-1}$ ), was calculated as follows:

$$BP = N (e^{\mu_c} - 1) \times V \times F$$

where N, bacterial counts (cells ml<sup>-1</sup>);  $\mu_c$ , mean bacterial growth rate in the control bottles; V, the bacterial cell volume of 0.146  $\mu\text{m}^3$  (Nagata & Watanabe 1990); and F, the C/V ratio of 120 fg C  $\mu\text{m}^{-3}$  (Nagata & Watanabe 1990).

## RESULTS

From June to October 2002, we detected 11 times of occurrence of bottom intrusion (Fig. 2). Among our sampling dates, 02 July, 2 August, and 9 October 2002 were during the occurrence of bottom intrusion (Fig. 2, Table 1). Our CTD monitoring data revealed that bottom intrusion also occurred on 18 June 2003 (data not shown). From late August to mid September, water temperature at 2 m depth increased from 23.8°C to 27.6°C, indicating the occurrence of *kyucho*, which was intrusion of warm, oligotrophic surface water from the south of the Bungo Channel to the west coast of Shikoku Island (Takeoka et al. 1993, Takeoka & Yoshimura 1988). In Uchiumi Bay, thermal stratification usually develops between May and September (Ichinotsuka et al. 2006, Katano et al. 2004, Katano et al. 2005). This was also the case during the sampling period for this study, though thermal stratification in May 2003 was still minor (Fig. 3).



Thus, we can divide the study period into stratified (from May to September) and well mixed (from October to March) periods.

Seasonally changing patterns were found in concentrations of DIN and DIP (Fig. 3b, c). High concentrations of these nutrients were detected in 20-50 m depth in July, August and October when bottom intrusion occurred (Figs. 2 and 3b, c). In October 2002, nutrient concentrations throughout the water column were high: 2.44 to 6.67  $\mu\text{mol N l}^{-1}$  (Fig. 3a), and 0.27 to 0.40  $\mu\text{mol P l}^{-1}$  (Fig. 3b). Such high concentrations of DIN and DIP continued during mixing period from October to March. In September 2002, concentrations of these nutrients were low throughout the water column, due to the intrusion of *kyucho* (Fig. 2).

A high concentration of DOC was detected in February 2003 in 0-15 m depth (176.7  $\mu\text{mol C l}^{-1}$ ) and it decreased to 50.4  $\mu\text{mol C l}^{-1}$  in April (Fig. 3d). The information about DOC concentration in the present study is limited. However, we continued to determine DOC concentration in 2003, 2004 and 2005 and observed no seasonal change in the vertical profile of DOC concentrations (unpublished observations).

Chlorophyll *a* concentrations ranged between 0.17 and 1.77  $\mu\text{g l}^{-1}$  during the investigation (Fig. 3e). The concentrations from August to December 2002 did not show

a clear vertical distribution, and cyclic changes between high and low concentrations were found from month to month (Fig.3e).

Bacterial cell counts ranged between  $0.4 \times 10^6$  cells  $\text{ml}^{-1}$  and  $2.7 \times 10^6$  cells  $\text{ml}^{-1}$  (Fig. 3f). Higher bacterial numbers were detected throughout the water column from June to September 2002 and from April to June 2003 (Fig. 3f). The period of high bacterial density roughly corresponded to the stratified period (Fig. 3a, f), otherwise the difference in bacterial density between 0-15m and 20-50m depth was not clear.

Bacterial growth rates (control treatment) at the 10 m depth in Uchiumi Bay ranged between 0.37 and 1.86  $\text{d}^{-1}$  (Fig. 4) and showed a clear seasonal change with relatively high growth rates during the stratified period (Figs. 3a and 4). In most cases, nutrients or glucose addition did not have an effect on bacterial growth rates, and a statistically significant stimulation of bacterial growth rates was only detected in the experiment of phosphate addition in January 2003 (Table 1).

There was no significant correlation between bacterial growth rates and each environmental variable (water temperature and concentrations of DIN, DIP, and chlorophyll *a*) examined in the present study. Seasonally changing pattern of bacterial production was almost identical to that of bacterial growth rates (data not shown). We tried Billen's approach (Billen et al. 1990) to examine the relationship between cell

density and production of bacteria, and found a significant logarithmic relationship between them (Fig. 5,  $r^2 = 0.78$ ,  $p < 0.001$ ,  $n = 13$ , slope = 0.34).

## DISCUSSION

Bacterial cell density is suggested to increase with the increase in bacterial production, implying that bacterial biomass at steady state is a direct function of substrate supply (Billen et al. 1990, Pace & Cole 1994). Previous studies have used Billen et al. (1990) to examine bottom-up control of bacterial biomass from the regression slope of bacterial biomass on bacterial production (Dufour & Torretton 1996, Pace & Cole 1994). Low slopes found in open ocean (e.g. 0.28) (Dufour & Torretton 1996) suggest that bottom-up control on bacterial biomass is weak, while high slopes found in productive waters (e.g. 0.7) (Billen et al. 1990) signify substantial bottom-up control on bacterial biomass. In the present study, the regression slope of bacterial cell density on bacterial production was found as 0.34, suggesting bottom-up control on bacterial abundance in Uchiumi Bay is weak relative to that of other waters (Billen et al. 1990, Pace & Cole 1994). In our previous study (Ichinotsuka et al. 2006), turnover rates of bacteria due to grazing by nanoflagellates and ciliates (1 to 16 % d<sup>-1</sup>) were seasonally

high, indicating seasonal importance of top-down control on bacterial abundance. This may be reason why we detected weak bottom-up control on bacterial abundance in the bay.

Most bacterial production rates in previous studies have been determined by the  $^3\text{H}$ -thymidine or  $^{14}\text{C}$ -leucine incorporation methods (Choi et al. 2001, Lee et al. 2001, Riemann et al. 2002, Vaque et al. 2001). We used a bottle incubation method because radioactive tracers are restricted from use in natural environments in Japan. Since bacterial growth rates are within the range of bacterial growth rates of previous studies (Table 2), bacterial growth rates determined in Uchiumi bay are comparable to those in other environments.

Previous studies have demonstrated nutrient (Caron et al. 2000, Hoch & Bronk 2007, Pomeroy & Diebel 1986, Sala et al. 2002) and organic matter (Carlson et al. 2002, Caron et al. 2000, Flaten et al. 2003, Sala et al. 2002) limitation on growth of marine bacteria. Caron et al. (2000) reported that the supplement of glucose to a water sample from the eutrophic waters of Georges Bank stimulated bacterial growth. Whereas, bacterial growth in water samples from oligotrophic waters of the Sargasso Sea was enhanced by a supplement of inorganic nitrogen and phosphorus. Bacterial growth rates of the present study in almost all cases were not stimulated by nutrient or glucose

addition (Table 1), though the water in the present study is oligo- to mesotrophic. Hence, our results suggest that bacterial growth rates of Uchiumi Bay is nearly saturated at ambient levels of nutrients and/or organic matter.

The bacterial-growth saturation by inorganic nutrients and/or organic matter (Fig. 4) seems to contradict the bottom up control of bacteria abundance (Fig. 5). One explanation for this is inappropriate use of carbon source in the experiment. The use of glucose as supply of organic matter to marine bacteria needs some caution. Recent studies have demonstrated that not all marine bacteria use glucose (Alonso & Pernthaler 2006, Alonso-Saez & Gasol 2007, Malmstrom et al. 2005). In Uchiumi Bay, one of the dominant bacterial phylogenetic group throughout the year is *Cytophaga-Flavobacterium* (unpublished observations), and it has been reported that the bacteria included in the group show active uptake of glucose at high concentrations (Alonso & Pernthaler 2006). Since glucose was added at a high concentration ( $30 \mu\text{mol C l}^{-1}$ ) in our nutrient amendment experiment, stimulation of bacterial growth rates would be expected. However, almost no response was found in bacterial growth rates (Table 1). Other organic substances, such as amino acids (Schweitzer & Simon 1995), might be stimulative for bacterial growth in Uchiumi Bay, though we did not examine them in the present study.

Our monthly monitoring data from 2001 to 2005 at the station showed significant positive relationship between bacterial cell density and chl. *a* concentration ( $p < 0.05$ ,  $n = 48$ , data not shown). This supports that bacterial abundance highly depends on the primary production. Thus organic matters derived from exudates of phytoplankton possibly control bacterial growth rates.

The other explanation for the contradiction is temperature. In the present study, bacterial cell densities and bacterial growth rates tend to be high during stratification period (Figs. 3 and 4), although the relationships were not significant as mentioned above. Our monthly monitoring data showed similar trend; Bacterial cell density was high during the warm water season, although significant relationship was not detected (data not shown). Therefore, the other possible factor to control bacterial abundance is water temperature.

Cross system analyses using data from various marine and freshwater ecosystems showed that bacterial abundance had a positive correlation with chlorophyll *a* concentration (Bird & Kalff 1984, Cole et al. 1988, Sommaruga & Robarts 1997). These findings suggest that bacterial abundance was mainly supported by DOC derived from phytoplankton. However, seasonal changes in vertical bacterial density of the present study (Fig. 4B) were different from those of chlorophyll *a* concentration (Fig. 4A) and

DOC concentrations, and we did not find any significant relationship between bacterial growth rates and chlorophyll *a* concentrations. These results suggest that DOC supply by phytoplankton was of minor importance for bacterial density in Uchiumi Bay.

Our previous study indicated that phytoplankton in Uchiumi Bay was subjected to systemic and/or growth rate limitations by N, P or Si (Hashimoto & Nakano 2003). Hence, we expected bacterial growth rates would have been stimulated by the addition of nutrients or organic matter. However, this was not the case (Table 1). In Uchiumi Bay, differences in the response to nutrient supply between large and small phytoplankton have been reported (Nakano et al. 2004). Major nutrient supply to the Bay is primarily due to bottom intrusion (Takeoka et al. 2000), and the high abundance of larger phytoplankton such as diatoms is detected when nutrients are supplied by bottom intrusion (Nakano et al. 2004). In contrast, the abundance of small phytoplankton such as picophytoplankton is high during warmer water temperature, independent of nutrient supply, due to the occurrence of bottom intrusion (Nakano et al. 2004). The responses of heterotrophic bacteria to nutrient supply or temperature changes have been observed to be similar to that of picophytoplankton (Nakano et al. 2004). It is likely that bacterial growth rates in Uchiumi Bay is nearly saturated at ambient levels of nutrients and/or organic matter, and that bacterial abundance is subject to relatively weak bottom-up

control. Although we did not find any significant relationship between temperature and bacterial abundance or the growth rates, bacterial abundance seasonally high during the thermally stratified period (Fig. 3 and our unpublished data). Thus, temperature possibly regulates them. Although we did not evaluate other organic matter for the experiment than glucose, probably other organic matter is a factor to regulate the bacterial abundance. Effect of temperature and other organic substrate should be further investigated to understand the bacterial dynamics in the sea.

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## Figure Captions

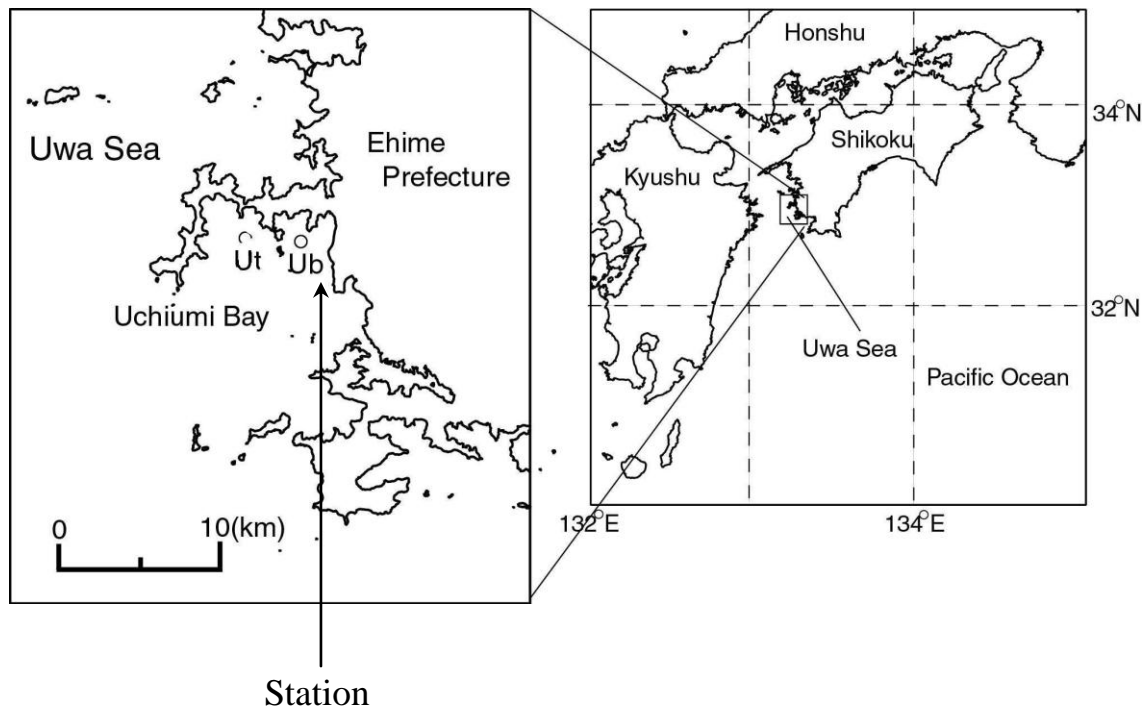
Fig.1. Locations of the sampling station (Stn. Ub) and temperature monitoring station (Stn. Ut) in Uchiumi Bay.

Fig.2. Changes in water temperature at 2- and 60 m depths at Stn. Ut. Arrows with characters indicate the occurrence of bottom intrusion.

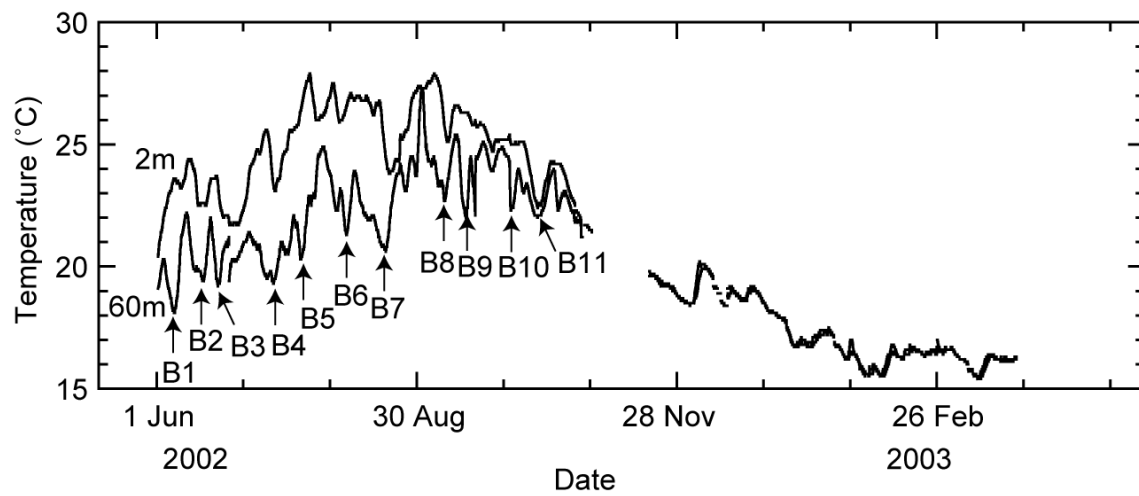
Fig.3. Seasonal changes in water temperature (panel a), dissolved inorganic nitrogen concentrations (DIN, panel b), dissolved inorganic phosphorus concentrations (DIP, panel c), dissolved organic carbon concentrations (DOC, panel d), chlorophyll *a* concentrations (chl. *a*, panel e), and bacterial cell densities (panel f),. White and black triangles in panel a show data from 0- and 50 m depths, respectively, and white circles and white squares in panels b, c, d, e, f, and g show mean values of samples collected from 0 – 15 m depth and 20 - 50 m depth, respectively.

Fig.4. Growth rates of bacteria obtained in the nutrient amendment experiments. Bars and error bars indicate mean values and standard deviations for four replications, respectively. Asterisk (\*) on the phosphate treatment in January shows significant difference ( $p < 0.05$ , Tukey's test after one-way ANOVA) among the treatment in January.

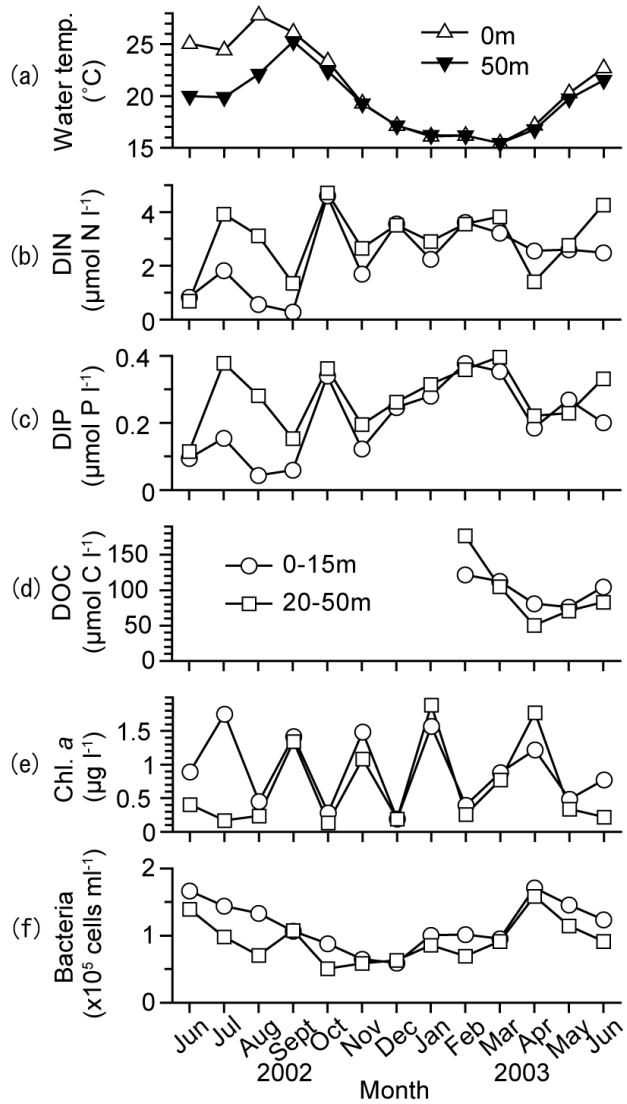
- 1 Fig.5. Logarithmic relationship between cell density and production of bacteria from
- 2 June 2002 to June 2003 in Uchiumi Bay. It should be noted that the bacterial
- 3 production was calculated from the growth rate and the cell density.
- 4
- 5



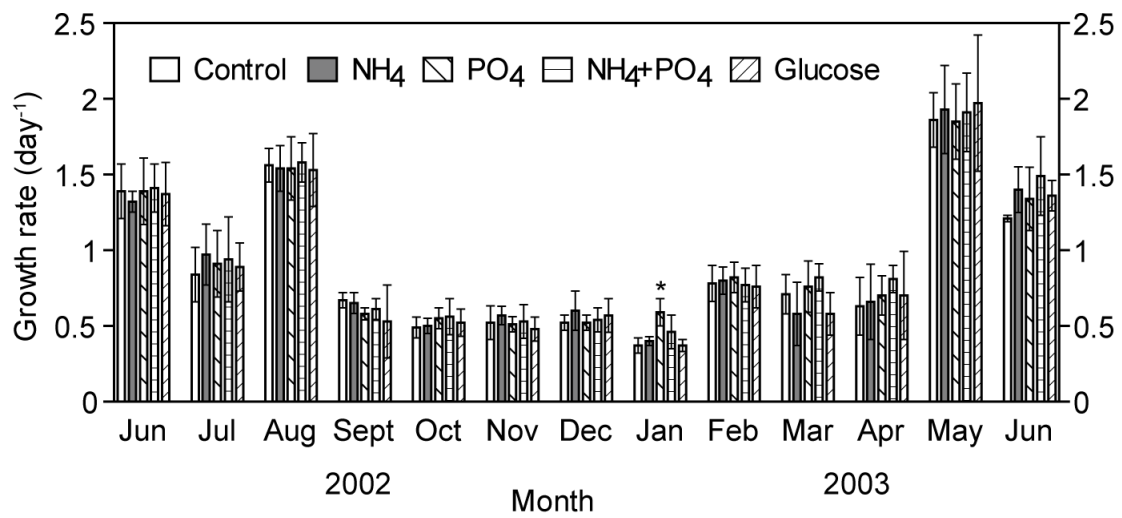
Ichinotsuka et al. Fig. 1



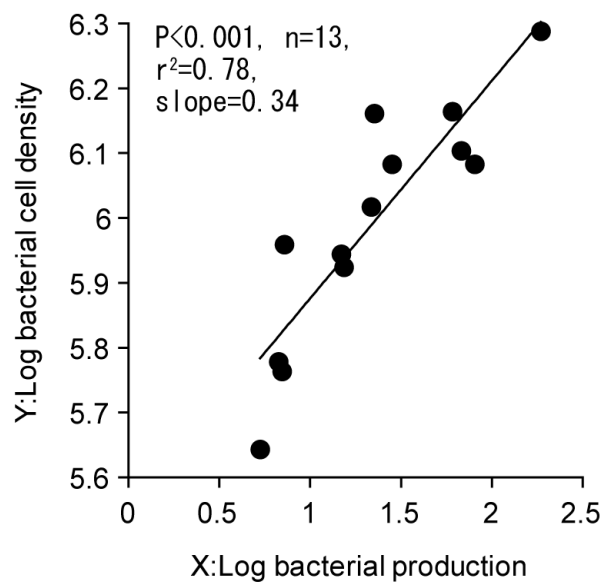
Ichinotsuka et al.      Fig. 2



Ichinotsuka et al. Fig. 3



Ichinotsuka et al. Fig. 4



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6 Ichinotsuka et al. Fig. 5



1 Table 1 Sampling dates and the days after the bottom intrusion.

Date	Days after the bottom intrusion	Event number* <sup>2</sup>
13 Jun 02	-	
12 Jul 02	1	B4
2 Aug 02	0	B6
11 Sep 02	-	
9 Oct 02	0	B11
8 Nov 02	ND* <sup>1</sup>	
10 Dec 02	-	
17 Jan 03	-	
13 Feb 03	-	
12 Mar 03	-	
16 Apr 03	ND* <sup>1</sup>	
20 May 03	ND* <sup>1</sup>	
18 Jun 03	0	_* <sup>3</sup>

2 \*<sup>1</sup>ND, not determined

3 \*<sup>2</sup> cf. Fig. 1

4 \*<sup>3</sup> Identified with other unpublished data

1

Table 2 Growth rates of bacteria in the literature and the present study.

Location	Growth rate (d <sup>-1</sup> )	Source
Catalano Balearic Basin, Mediterranean Sea	0.19 - 1.54	Sala et al. (2002)
Funka Bay	0.02 - 2.57	Lee et al. (2001)
NW Mediterranean Sea	0.01 - 0.35	Vaque et al. (2001)
Oregon Coast	0.01 - 0.31	Sherr et al. (2001)
Schelde estuary	0.1 - 1.2	Hamels et al. (2001)
Scripps Pier	0.3 - 2.2	Riemann et al. (2002)
Southern Ocean	0.02 - 0.78	Church et al. (2000)
West of Barbados, Caribbean Sea	0.16 - 1.46	Choi et al. (2001)
Uchiumi Bay	0.37 - 1.86	This study

2